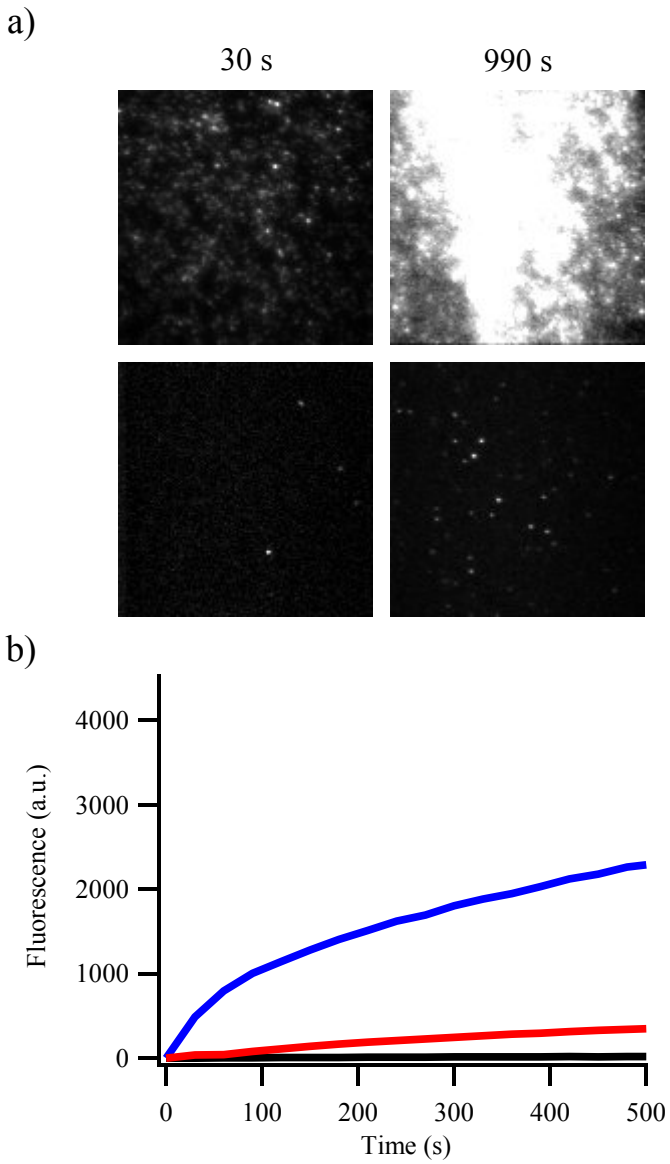
Supporting Material

to

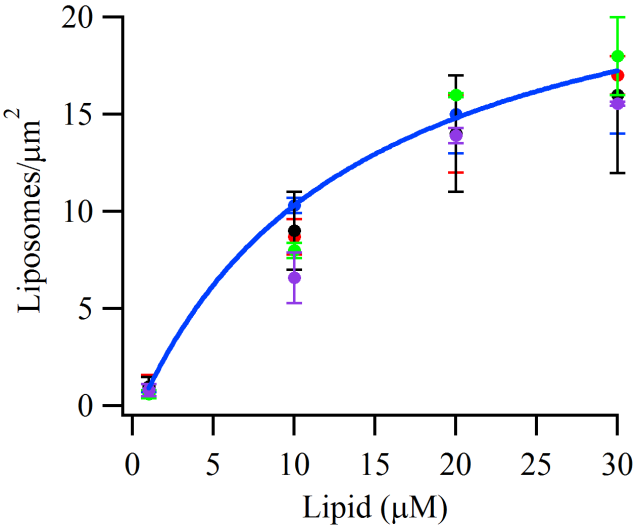
High Cholesterol Obviates a Prolonged Hemifusion Intermediate in Fast SNARE-Mediated Membrane Fusion

Alex J. B. Kreutzberger,[1] Volker Kiessling,[1] and Lukas K. Tamm[1,*]

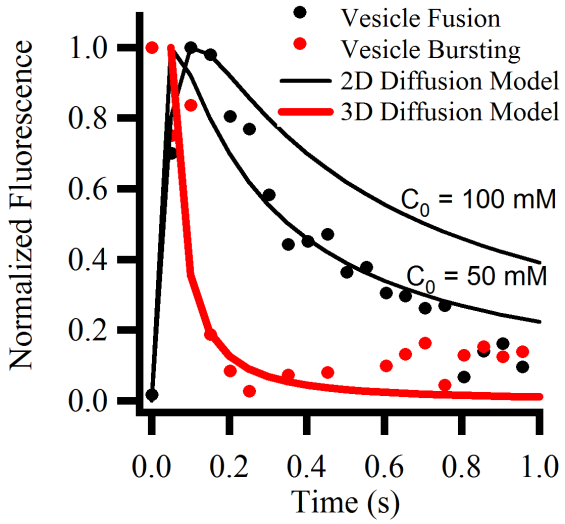
[1]Center for Membrane Biology and Department of Molecular Physiology and Biological Physics, University of Virginia, Charlottesville, Virginia



Supporting Figure S1: Docking controls of 5 μM lipid of syb2 proteoliposomes to a planar supported bilayer. Conditions shown are for 0 mol% cholesterol in the planar bilayer and 54:20:5:20:1 bPC:bPE:bPS:chol:Rh-DOPE in the syb2 proteoliposome. **a)** Representative images of docking 30 seconds after assay has begun (left column) and well after saturation has occurred (right column). High docking is observed (top row) in normal conditions and no docking is observed when the syb96 inhibitor peptide was added as a control (bottom row). **b)** Fluorescence in the TIRF field over time indicating syb2 proteoliposomes interacting with the planar supported bilayer. High syb2 proteoliposome docking is observed when both t-SNARE and v-SNAREs are present (blue), but very little docking is observed in the absence of t-SNAREs (red) or in the presence of the syb96 peptide (black).



Supporting Figure S2: Docking of syb2 proteoliposomes on acceptor t-SNARE containing planar supported bilayers with different concentrations of cholesterol: 0 mol% (black), 10 mol% (red), 20 mol% (blue), 30 mol% (green), and 40 mol% (purple). Error bars are standard deviations from 3 to 4 experiments. The data are plotted as number of syb liposomes bound per μm^2 versus added syb proteoliposome lipid concentration. The blue curve shows the best fit of a Langmuir isotherm to the 20% cholesterol data.



Supporting Figure S3: Decay of sulforhodamine B fluorescence from a single vesicle due to diffusion into the cleft between the supported membrane and the quartz substrate after controlled fusion of the vesicle with the supported membrane (black data points) and from a single vesicle into the surrounding buffer after bursting of the vesicle on a lipid monolayer of bPC:chol (80:20) (red data points). The solid lines are simulations of a 2D dye diffusion model (black lines) and a 3D dye diffusion model (red line) according to the theory of Wang et al. (1) with typical diffusion coefficients as described below.

Content transfer from a vesicle into the cleft between a planar supported bilayer and its substrate can be modeled by two-dimensional diffusion from a point source by calculating the absolute integrated fluorescence intensity within a cylindrical volume of radius R centered on the release point and height h in cylindrical coordinates:

$$F(t) = A \int_0^R 2\pi\rho d\rho \int_0^h C(\rho,z,t)\phi_{rel}(\rho,z,t)I_{laser}(z)dz \qquad \text{(S1)}$$

The radial coordinate centered on the z axis is ρ and z = 0 is defined as the substrate surface, C is the concentration of the dye as a function of position and time, and A is an unknown proportionality constant. $I_{laser}(z)$ is the intensity profile from TIRF excitation which is $I_{laser}(z) = I_0\, exp(-z/z_0)$, where z_0 is the characteristic decay length of the evanescent field, i.e., 102 nm in our case. ϕ_{rel} is the relative concentration dependent quantum yield of the fluorophore, which is determined by measuring the fluorescence intensity per molecule across a range of dye concentrations on the TIRF microscope (1,2). The fit of the exponential decay of sulforhodamine B on our set up was $\phi_{rel} = e^{-74C(\rho,z,t)}$. The model for two-dimensional diffusion of the dye in the cleft is

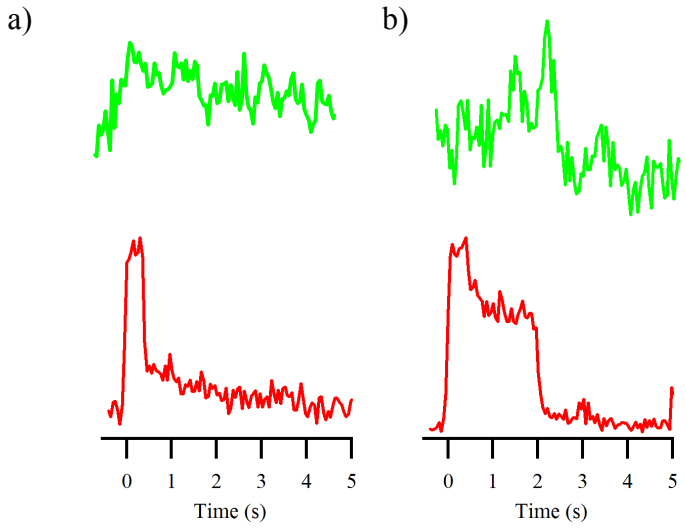
$$C(\rho,t) = \frac{C_0 V_{ves}}{4\pi h D t} e^{-\rho^2/4Dt} \qquad \text{(S2)}$$

When a vesicle bursts, the dye diffuses in 3D into a half-space above the vesicle and the corresponding model for three-dimensional diffusion

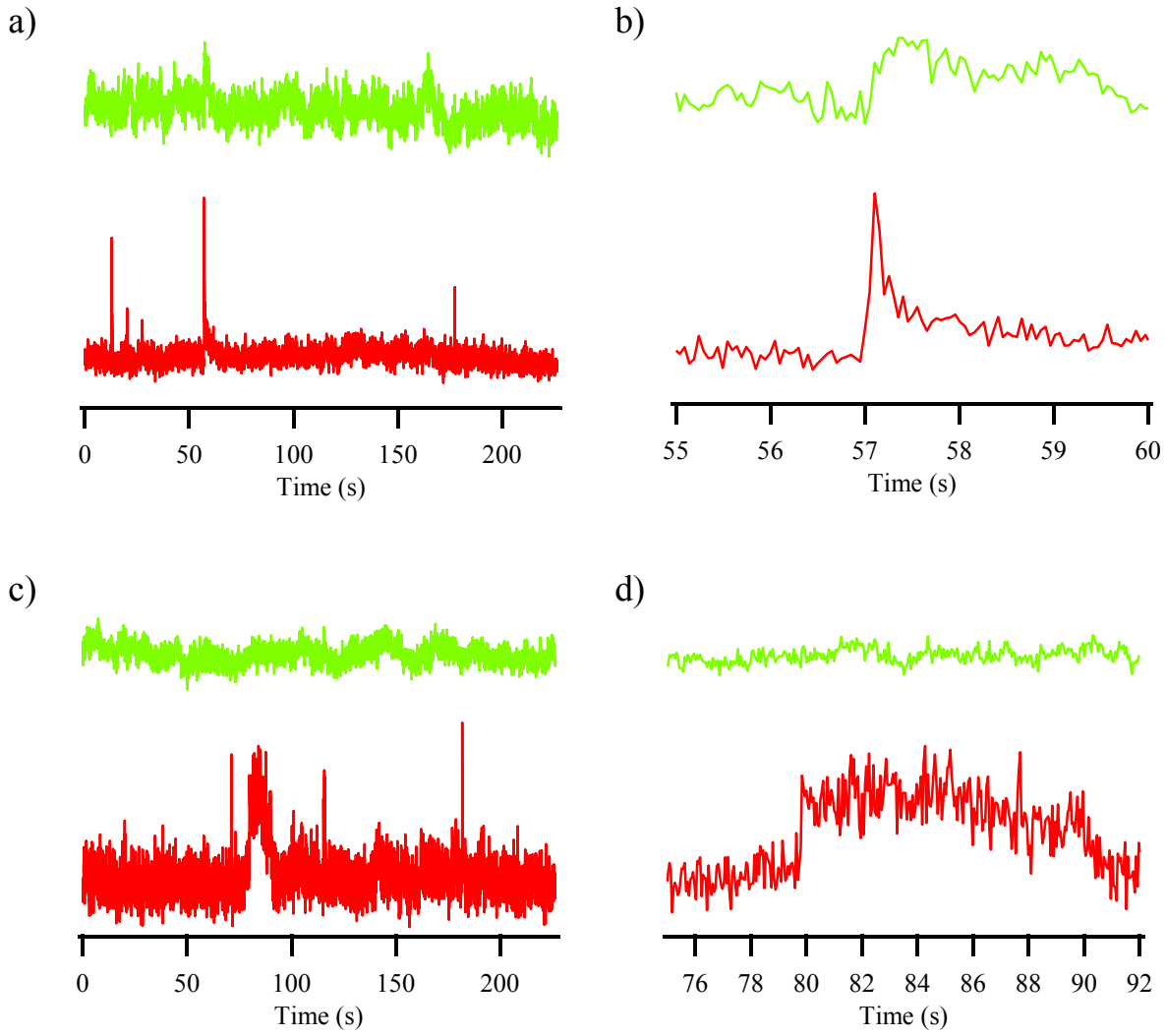
$$C(\rho, z, t) = \frac{2C_0 V_{ves}}{(4\pi Dt)^{3/2}} e^{-(\rho^2+z^2)/4Dt} \quad \text{(S3)}$$

needs to be inserted into equation S1. Our initial concentrations C_0 of sulforhodamine B in the vesicle were 100 mM in both cases assuming that 100% of dye is encapsulated in vesicles of volume V_{ves} = 3.88 x 10^{-20} L for a 42 nm liposome. *D* in equations S2 and S3 are the solution diffusion coefficients of sulforhodamine B, i.e. 4.0 x 10^{-10} m^2/s (3).

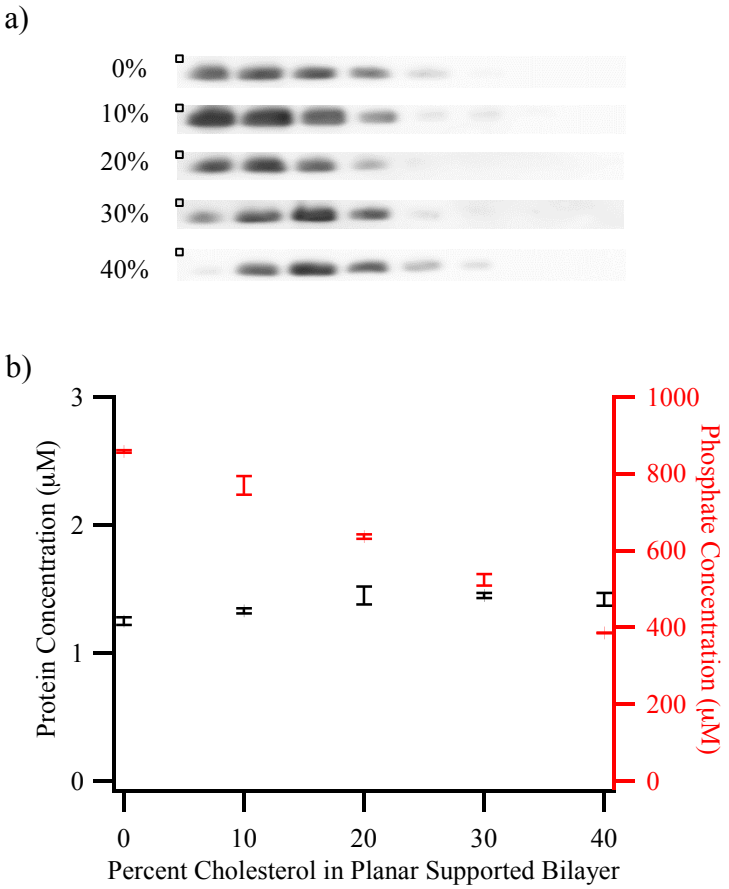
As done previously (2), the fluorescence intensities were normalized and the decay profiles were used to distinguish between two- and three-dimensional diffusion. Mathematica was used to perform the numerical integrations of the normalized fluorescence intensity for each diffusion model. The integration in ρ went from 0 to *R* = 625 nm. The integration in h went from 0 to 2 nm for 2D diffusion in the cleft and from 6 nm to 2 μm for 3D diffusion into the half-space above the vesicle, respectively. The solid lines are simulations using these models with 100 mM and 50 mM sulforhodamine B as indicated. The reason that the fit using C_0 = 50 mM is better may be rationalized by the fact that the dye is not fully encapsulated during preparation on the gel filtration column.



Supporting Figure S4: Two rarely observed types of events from the single vesicle fusion assay. **a)** A single event of full fusion, in which the content dye did not diffuse away. **b)** two-step hemi- to full fusion event, in which content dye returned to base-line level at the full fusion step. This behavior is as frequently observed as the behavior shown in Fig. 1, example IV, but both types are rare, accounting combined for not more than 2% of all observed fusion events. The color scheme is the same as in Fig. 1.

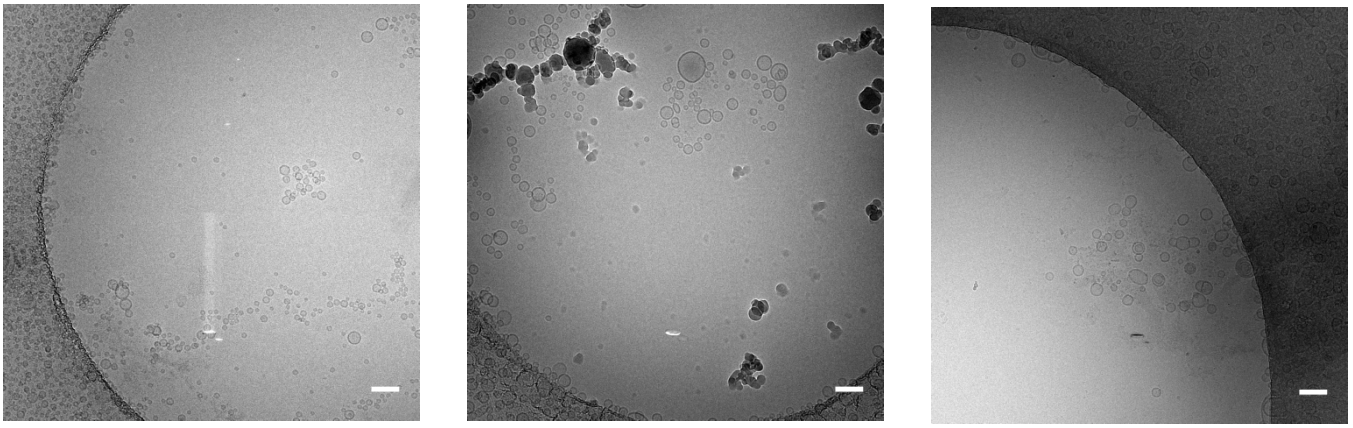


Supporting Figure S5: Characteristic traces of whole recording times of a fusion (**a**) and a docking (**c**) event. Panels **b** and **d** show respective events of **a** and **c** on expanded time scales. Note that some photo-bleaching of the membrane dye takes place after ~9 seconds in the docking event (**d**).



Supporting Figure S6: a) Western blots of SNAP-25 from a co-floatation assay indicating full insertion of the acceptor t-SNARE complex for all cholesterol compositions used in the planar target membranes. Lanes from left to right indicate fractions from the top to the bottom of the gradient. **b)** Protein and phosphate concentrations of proteoliposomes used to form the outer leaflets of the planar supported target membranes as determined by the BCA and modified Bartlett assays, respectively. The results are from four independent reconstitutions of acceptor t-SNARE complex proteoliposomes.

a) b) c)



Supporting Figure S7: Representative overview cryo-electron microscopy images for **a)** 0, **b)** 20, and **c)** 40 mol% cholesterol in syb2 proteoliposomes.

Supporting References

1. Wang T, Smith EA, Chapman ER, Weisshaar JC. 2009. Lipid mixing and content release in single-vesicle, SNARE-driven fusion assay with 1-5 ms resolution. *Biophys J.* 96:4122-4131.

2. Rawle RJ, van Lengerich B, Chung M, Bendix PM, Boxer SG. 2011. Vesicle fusion observed by content transfer across a tethered lipid bilayer. *Biophys J.* 101:L37-39.

3. Gendron PO, Avaltroni F, Wilkinson KJ. 2008. Diffusion coefficients of several rhodamine derivatives as determined by pulsed field gradient-nuclear magnetic resonance and fluorescence correlation spectroscopy. *J Fluoresc.* 18:1093-1101.